UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/027,089	02/02/1998	FRANK H. PRORTUGAL	CAB-001	2654

7590 08/05/2002

MILLEN,WHITE,ZELANO & BRANIGAN,P.C.
2200 Clarendon Boulevard, Suite1400
Arlington, VA 22201

EXAMINER	
SOUAYA, JEHANNE E	

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 08/05/2002 27

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/027,089	Applicant(s) PRORTUGAL, FRANK H.	
	Examiner Jehanne Souaya	Art Unit 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 19-24 and 26-35 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) 25 and 37-45 is/are allowed.

6)☒ Claim(s) 19-24 and 26-35 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Continued Prosecution Application

1. The request filed on April 16, 2002 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/027,089 is acceptable and a CPA has been established. An action on the CPA follows.

2. Currently, claims 19-35 and 37-45 are pending in the instant application. Applicant's amendments filed 4/16/2002 and 6/5/2002 have been entered. All the amendments and arguments have been thoroughly reviewed but are deemed insufficient to place this application in condition for allowance. Any rejections not reiterated are hereby withdrawn. The following rejections are either newly applied or are reiterated. They constitute the complete set being presently applied to the instant Application. This action is NON-FINAL.

3. The rejection under 35 USC 102 (b) of claim 25 as being anticipated by accession number X80728 is moot due to the amendment to claim 25.

4. The provisional rejection under 35 USC 102 (e) based on copending application 09/027,439 is moot as the '439 application is abandoned.

5. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Drawings

6. New formal drawings are required in this application because the specification contains drawings (figures 1 and 2) embedded in the text. Such a format is not acceptable as the drawings should be placed on separate sheets of paper and are not part of the text of the invention. It is also noted that a flow diagram is present on the last page of the specification. This diagram is not designated as a figure, but is considered a figure and should also be included separately from the text of the invention. Further, a "Brief Description of the Drawings" which explains the figures is not present in the specification. Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the Patent and Trademark Office no longer prepares new drawings. The corrected drawings are required in reply to the Office action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance.

New Grounds of Rejection

Claim Rejections - 35 USC § 103

7. Claims 19, 23, 24, and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Hammond et al (US Patent 5,374,718: Dec. 20, 1994) and Hogen (US Patent 5,714,321, 102(e) date: 2/22/94) and Dyson, N.J. (Essential Molecular Biology Vol. II: A Practical Approach, chapter 5, pages 111-156, Brown, T.A. ed. Oxford University Press, Oxford, 1992) and Anderson (Gene Probes 2: Hybridization Strategy, pp 1-29, Oxford University Press,

New York, 1995) in view of Cilia et al (Mol. Biol. Evol., vol. 13, pp 451-461, 1996) and Accession number X80728, 3/29/1996).

The claims are drawn to a method of discriminating between or among species of Shigella and E. coli in a sample containing organisms of one or mor taxonomic groups by selecting a probe from an operon common to two or more organisms of the taxonomic groups, wherein the probe contains one or more base mismatches and wherein the probe is capable of discriminating between organisms by hybridization at two or more wash temperatures at or above the probes calculated or experimentally determined Tm, hybridizing the probe to the nucleic acid in the sample, and determining the presence or absence of hybridizing nucleic acid.

Methods of using probes to identify or differentiate closely related organisms was well known in the art at the time of the invention, as well as manipulations of reaction conditions to increase stringency, as can be exemplified by the teachings in the following references. Hammond teaches hybridization assay probes specific for chlamydia pneumoniae which can distinguish *C. pneumoniae* form its most closely related taxonomic or phylogenetic neighbors (see col. 3, lines 35-40). Hammond teaches obtaining suitable probes for detection and discrimination. Hammond generally teaches that all prokaryotic organisms (except for viruses) contain rRNA genes. Hammond teaches that variable regions of rRNA sequences from the 16S rRNA of *C. pneumoniae* were identified by sequencing the rRNA of *C. pneumoniae* and its closely related phylogenetic neighbors and aligning the sequences to reveal areas of maximum homology and also alignment for regions of sequence variation (col. 3, lines 41-55). For

construction of suitable probes, Hammond teaches that first, the stability of the probe:target nucleic acid should be chosen to be compatible with assay conditions, ie: hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures (col. 4, lines 51-65). Hammond teaches that ionic strength and incubation temperature under which a probe will be used, should be taken into account. Hammond teaches that incubation at temperatures below the optimum Tm may allow mismatched base sequences to hybridize and can therefore result in reduced specificity (col. 5, lines 8-15). Hammond further teaches that it is desirable to have probes which hybridize only under conditions of high stringency.

Hogan also teaches a method for preparing probes for use in qualitative and quantitative assays wherein the probes are capable of detecting and differentiating between eubacteria (see abstract). Hogan also teaches the hybridization of *E. Coli* probes to closely related organisms such as *Shigella boydii, Sh. flexneri, Sh. dysenteriae, and Sh. sonnei* (see col. 52, table 54). Hogan also generally teaches hybridization strategies, including variations in temperature, probe length, probe composition, and ionic strength in methods of identification of target nucleic acids (cols 7-11) and specifically points out that use of temperatures below the optimum (Tm) may allow mismatched base sequences to hybridize and can therefore result in reduced specificity (col. 10, lines 21-24). Hogan also specifically teaches using filter hybridization methods, and the use of rRNA sequences in distinguishing between eubacteria (cols 1 and 2).

Anderson teaches hybridization strategies in constructing probes for methods of screening and identification. Anderson teaches factors affecting the rate of hybridization and the stability

of hybrids, (p. 3-13) including probe length, composition, and temperature. Anderson specifically applies these manipulations to filter hybridization. Anderson also specifically teaches that to detect closely related family members, it is better to use stringent hybridization conditions followed by stringent washing conditions (for example, from the teaching of the previous three references, the ordinary artisan would be taught that such a condition could involve high temperature, etc) (p. 13, last sentence).

Dyson teaches that nucleic acid hybridization is an important component of many molecular biology techniques, and that specifically, filter hybridization methods exploit the specificity of molecular hybridization for the detection of rare sequences in a complex mixture (see p. 111, first paragraph). Dyson teaches different methods for immobilization of nucleic acids on filters (pp 111-132) and teaches factors affecting hybridization of nucleic acids (pp 132-151). Dyson teaches that such factors include Tm, base composition, mismatching (p 133), and ionic strength affect hybridization. Dyson teaches that filter hybridization involves three basic steps: pre-hybridization, hybridization, and washing (p. 137, section 3.4). Dyson teaches that after hybridization, the filter is washed to remove the probe. Dyson teaches that short DNA duplexes have a reduced melting temperature and the Tm of oligonucleotide probes can be calculated, although the actual Tm should be determined experimentally (see p 146). Dyson specifically teaches that oligonucleotides are hybridized at a temperature between 5 and 10 degrees below the Tm for 14-48 hours and that filters are then washed four times *at* the hybridization temperature (see p. 147, lines 1-3). Dyson teaches that often, such a wash is

enough, however Dyson teaches that if the filters still show considerable activity above background, the wash temperature should be increased by 2-3 °C and the wash should be repeated.

Although neither Hammond, Hogan, Dyson or Anderson teach using the probes of the instant invention, Cilia et al teaches sequence heterogeneities among 16S RNA sequences of E. Coli and Shigella (see abstract, and figure 3) and teaches nucleotide differences among Eubacteria by showing a line up of regions from 16S genes across species levels, showing the nucleotide sequence similarities and differences. Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to construct the DNA sequences of the claimed invention for the use of probes and primers that could distinguish *Shigella* from *E. Coli*. Methods of distinguishing between different eubacteria using probes and primers that target regions of similarity and differences were readily known in the art at the time of the invention and is exemplified by the Hogan patent. The ordinary artisan would have been motivated to construct probes and primers of the claimed invention to identify and differentiate *E.coli* from *Shigella* as Cilia teaches how closely related the two genus of bacteria are (see Fig 1). Further, the sequence of SEQ ID NO 4 was known in the art at the time of the invention. [Applicant was faxed a copy of the results of a sequence search which discloses the complete nucleotide sequence of SEQ ID NO 4.] As the sequences of the 16S rRNA and rDNA sequences of the *Shigella* species and *E.coli* sequences were known at the time of the invention, it would have been obvious for the ordinary artisan to construct probes and primers to regions of

variability to be able to differentiate the closely related bacteria. Such methods were readily known in the art as is shown by the large amount of literature available in the art that identifies regions of variability among closely related bacteria for the purpose of constructing probes and primers useful in methods of differentiation.

It would have further been prima facie obvious to one of ordinary skill in the art to raise the temperature of the wash step to achieve maximum specificity and selectivity as Dyson teaches that the temperature of the wash step can be varied by incrementally increasing the temperature. Dyson also provides examples of lengths of probes as well as suggested hybridization and wash temperatures (see table 2, p. 147). In each case, the wash temperature is above the hybridization temperature. Therefore, although Anderson and Dyson teach <u>hybridizing</u> 5-10 degrees *below* the Tm of the probe, Dyson teaches <u>washing</u> above the hybridization temperature and that the wash temperature can be increased by 2-3 degrees. With such a teaching, and the examples in table 2, it would have been readily apparent to one of ordinary skill in the art to increase wash temperatures by 2-3 degrees at a time, and repeat as needed until suitable hybridization had occurred. It would have further been prima facie obvious to one of ordinary skill that because Dyson teaches *suggested* conditions and teaches that manipulations of conditions, such as wash temperature, can be performed to achieve the desired result, a certain amount of manipulation of conditions (such as changing salt concentration, varying temp of both hybridization and washing steps) could be necessary. As the level of skill in the art regarding hybridization of oligonucleotides is very high, the ordinary artisan would have considered that

the identification of optimum Tm for washing is a matter of routine optimization and that while one would initially wash at Tm below the Tm of the probe, where such conditions are insufficient to distinguish, the ordinary artisan would know to adjust the conditions, either by increasing the temperature or adjust the buffer (ie: salt concentration).

It is noted that claim 19 was amended to recite "at least one of which is above the oligonucleotide's calculated or experimentally determined Tm", however the claim amendments still do not overcome the teachings of the prior art because the method does not specifically recite a positive process step that includes washing above the calculated or experimentally determined Tm of the probe. A probe's Tm indicates the temperature at which half of the probes remain hybridized. Therefore, the property of the probe recited in step (a) does not overcome the teachings of the prior art because it would be expected that above the Tm of the probe, some of the probes will remain hybridized to the specific target for which they are designed to hybridize to.

With regard to claims 23, 24, and 26, it is noted that the claims recite using an oligonucleotide "comprising a sequence of SEQ ID NO 4". As a sequence "comprising SEQ ID NO 4" was known in the art at the time of applicant's invention (see Accession number X80728, which teaches that SEQ ID NO 4 is identical to a sequence within the *E. coli* rrnE gene) to be identical to a region for the *E. coli* rrnE gene one of ordinary skill in the art would have been motivated to use a sequence comprising SEQ ID NO 4 to specifically detect *E. coli*.

Note: the rejection to claims 19, 23 and 24 can be overcome by reciting a positive wash step above the probes' Tm. The rejection to claim 26 can be overcome by reciting "an oligonucleotide consisting of the sequence of SEQ ID NO 4".

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 19-24 and 26-35 are rejected under 35 U.S.C. 112, second paragraph, as failing to set forth the subject matter which applicant(s) regard as their invention. Evidence that claims 19-24 and 26-35 fail(s) to correspond in scope with that which applicant(s) regard as the invention can be found in Paper No. 15 filed 1/22/2001. In that paper (page 5, last para), applicant has stated "the invention of Portugal teaches that duplexes formed from oligonucleotides should be washed at temperatures that either at the Tm [of the oligonucleotide] or above it", and this statement indicates that the invention is different from what is defined in the claim(s) because the invention as presently claimed does not provide any positive method or process steps of "washing at temperatures that are either at the Tm [of the oligonucleotide probe] or above it". This criticality in applicant's invention is reiterated in paper No. 25, filed 4/16/2002 (see p. 6, paragraph 5).

10. Claims 19-24 and 26-35 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps.

See MPEP § 2172.01. The omitted steps are: "washing the hybridized oligonucleotide and nucleic acid at two or more wash temperatures, at least one of which is above the oligonucleotide's calculated or experimentally determined Tm" (for claim 19) and "washing the hybridized oligonucleotide and nucleic acid at two or more wash temperatures at or above the oligonucleotide's calculated or experimentally determined Tm" (for claim 26).

Note: Applicant can overcome the rejection made under 35 USC 112/second paragraph by reciting the indicated omitted method steps.

Conclusion

11. Claims 25 and 37-45 are allowable over the cited prior art.

12. It is noted that applicant has not addressed the exminer's concerns regarding the organization of the specification, set forth in the office action mailed 9/07/1999.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jehanne Souaya whose telephone number is (703)308-6565. The examiner can normally be reached Monday-Friday from 9:00 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jehanne Souaya

Jehanne Souaya
Patent examiner
Art Unit 1634

July 15, 2002